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Short communication

Response of the coagulation system after application of hemostatic dressings in an animal model

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Abstract

The objective of this study was to determine the response of hemostatic dressings. Coagulation and fibrinolytic systems, red blood cell parameters, platelet and leukocyte counts were evaluated after the application of hemostatic dressings: QuikClot, Chitoauze and Celox gauze. The experiment was performed on ten pigs.

Key words: hemorrhage, hemostatic dressing, blood vessels, pig

Introduction

Excessive hemorrhaging is the most frequent cause of death in combat and massive hemorrhaging quickly leads to hypovolemic shock which can result in death. Hemostatic dressings are effective in hemorrhage control but their influence on the living organism is not clear. Hemostatic agents are used in both military and civilian medicine during intervention and rescue operations (Ran et al. 2010, Travers et al. 2012). The effectiveness of hemostatic dressings such as QuikClot, Chitoauze and Celox gauze has been used in combat and in laboratory analyses, but there is still scanty information (Rall et al. 2013).

Materials and Methods

This study was approved by the Institutional Animal Care and Use Committee and all animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals.

The experiment was performed on 10 Polish Large White female pigs with a body weight of 30 kg. The animals were premedicated with azaperone (Stresnil) at 2.0 mg/kg BW IM and atropine (Atropinum Sulfuricum) at 0.05 mg/kg BW IM. General anesthesia was induced with ketamine (Bioketan) at 8 mg/kg BW IM and maintained with thiopental administered intravenously to effect. Butorphanol

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Table 1. Variance of samples ATIII and TAT.

Sampling time	Number of samples ATIII	Variance ATIII	Number of samples TAT	Variance TAT
0 min	10	37.21	10	346991.61
15 min	10	135.75	10	811400.44
30 min	8	342.30	8	2214683.33
60 min	8	81.29	8	10438076.49
12 h	5	121.20	5	5685065.20
24 h	4	61.67	4	16603195.19

Table 2. Pearson correlation coefficient, the degree of linear dependence between ATIII and TAT. The positive linear correlation between two variables is the most prominent in samples collected 30 minutes after application of hemostatic dressings.

Sampling time	Sample	ATIII	TAT	Correlation	Sampling time	Sample	ATIII	TAT	Correlation
0 min	1	118	2054	-0.220577064	60 min	1	95	0	0.17547415
	2	98	1899			2	0	5623	
	3	98	2632			3	85	6985	
	4	100	3000			4	87	13666	
	5	98	2543			5	95	6527	
	6	102	2635			6	112	8236	
	7	101	3928			7	101	13536	
	8	108	2595			8	94	5926	
	9	105	1925		12 h	1	86	5984	0.23831511
	10	98	2002			2	74	10532	
15 min	1	118	3696	0.092678031		3	94	7021	
	2	0	2566			4	104	12321	
	3	98	0			5	91	10562	
	4	78	4569		24 h	1	89	4222	-0.2946802
	5	93	2987			2	82	15586	
	6	108	2896			3	98	9561	
	7	105	5089			4	81	8256	
	8	110	2899						
	9	94	3892						
	10	99	2222						
30 min	1	101	6952	0.59225042					
	2	0	0						
	3	93	3256						
	4	65	5621						
	5	0	3652						
	6	107	2928						
	7	0	0						
	8	112	3241						

(Butomidor) was administered as the analgesic drug at 0.2 mg/kg BW IV. Blood samples were collected from all animals before anesthesia and 15, 30 and 60 minutes, 12 and 24 hours after the application of the hemostatic agent.

A complete blood count (CBC), thrombin-antithrombin complex (TAT) concentrations, prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), fibrinogen concentrations, antithrombin activity (AT III) and D-dimer concentrations were determined in laboratory analyses. CBC was determined in samples of whole blood collected into EDTAK_2 tubes in the Siemens ADVIA 2120i hematology analyzer by flow cytometry and the laser light scattering technique. TAT was determined in EDTA-treated plasma using the Biotek ELISA microplate reader, Cusabio ELISA kit and the porcine thrombin-antithrombin (TAT) complex. PT, APTT, TT, Fibrinogen, AT III and D-dimer were measured in citrated plasma using the Bio-Ksel Coag-Chrom 3003 coagulation analyzer and the Bio-Ksel System.

Results

A considerable decrease in RBC counts and hematocrit (HCT) levels, a minor drop in hemoglobin (HGB) concentrations and an increase in MCV and RDW values were observed in all analyzed samples. Twelve hours after the application of the hemostatic agent, MCV was restored to its initial level or was even lower. Thrombocytopenia was observed in the first hour. An increase in PLT counts and a significant increase in PDW were reported after 12 and 24 hours.

PT and APTT were prolonged 30 minutes after the beginning of treatment, after which a successive drop in both parameters was noted. TT was initially prolonged and it decreased over time. Fibrinogen concentrations increased in all samples. D-dimer concentrations increased substantially after the third collection of blood samples. Considerable changes in AT III activity and TAT concentrations were observed (Table 1.). A significant drop in AT III activity and an increase in TAT concentrations were reported in all samples (Table 2).

Discussion

Two coagulation cascade pathways, the intrinsic pathway and the extrinsic pathway, can be evaluated with the use of three basic tests – APTT, PT and TT. In this experiment, prolonged PT and APTT were observed 30 minutes after the beginning of treatment, after which a successive drop in both parameters was noted. Similarly to PT and APTT, TT was also initially prolonged, and it decreased over time. The TT test measures the conversion of fibrinogen to fibrin, which is determined by the presence and activity of thrombin inhibitors and by fibrinogen concentrations (Li et al. 2013). In this experiment, fibrinogen concentrations increased in all samples and at all-time intervals between sample collection and analysis; this could be attributed to the observed rise in fibrinogen levels. The activation of the fibrinolytic system and fibrinogen degradation resulted in shorter TT. Normal fibrinogen levels are noted after surgery and in sepsis when initial fibrinogen concentrations are elevated (Levi et al. 2006). In this study, high fibrinogen levels were indicative of inflammation. Significant changes in AT III activity and TAT levels were observed. AT III activity decreased and TAT concentrations increased during the experiment. Elevated TAT concentrations and suppressed AT III activity are signs of thrombogenesis. Very high levels of TAT could imply that the described processes were accelerated by the administration of procoagulants (Marcucci et al. 2006). Thrombocytopenia was observed in the first hour after the application of the hemostatic agent. A significant increase in PDW indicates that platelets were produced rapidly, which led to the movement of small platelets to the peripheral blood, and a numerous platelet aggregates were formed. Posthemorrhagic anemia was reported in all cases. Twelve hours after the application of the hemostatic agent, MCV was restored to its initial level or was even lower.

The results of this study can be used in future investigations of changes in the blood in a model species for comparative studies of hemostatic dressings.

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